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Probing the Hydrophobic Sites on the Surface of Serum Albumins Using Bromophenol Blue-Induced Fluorescence Quenching: A Comparative Study

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Hydrophobic sites on the surface of proteins are believed to play important roles in various biological phenomena including ligand binding to proteins. Various hydrophobic ligands, which bind to the same protein, may share these sites. In an earlier study, bromophenol blue (BPB) has been shown to bind to hydrophobic sites on the surface of serum albumin. In order to compare these hydrophobic sites on different serum albumins, we studied tryptophan fluorescence quenching of different serum albumins induced by BPB binding. All albumins produced a fluorescence spectra in the wavelength range 300-400 nm with emission maxima around 332-340 nm when excited at 280 nm. Addition of BPB to these solutions resulted in the decrease in fluorescence at the emission maxima. Different serum albumins showed differences in the magnitude of quenching when studied up to BPB/albumins molar ratio of 1:1. The fluorescence data were analyzed using two different methods and the values of binding parameters were determined. Different values of association constant ranging from $2.1 \pm 0.3 \times 10^5$ M^{-1} to $1.5 \pm 0.2 \times 10^7$ M^{-1} were found for different serum albumins, whereas the number of high affinity binding sites was determined to be 0.64 ± 0.02 - 1.22 ± 0.04. These results suggest micro-environmental changes in the hydrophobic sites in these albumins.